CHAPTER IV

RESULTS

Preparation of *H. sabdariffa* aqueous extract

Two kilograms of dry calyx of *H. sabdariffa* were used in this study. Following the extraction process, 494.85 g of the aqueous extract was obtained. Thus, percentage yield of the extract was 24.74 % w/w.

Preliminary identification of *H. sabdariffa* aqueous extract

1. Determination of chromatographic fingerprint of *H. sabdariffa* aqueous extract using HPLC.

The chromatographic fingerprint of *H. sabdariffa* aqueous extract was shown in figure 4. There were 3 chromatographic peaks shown at the retention time of 2.386, 2.813 and 6.030 minutes.

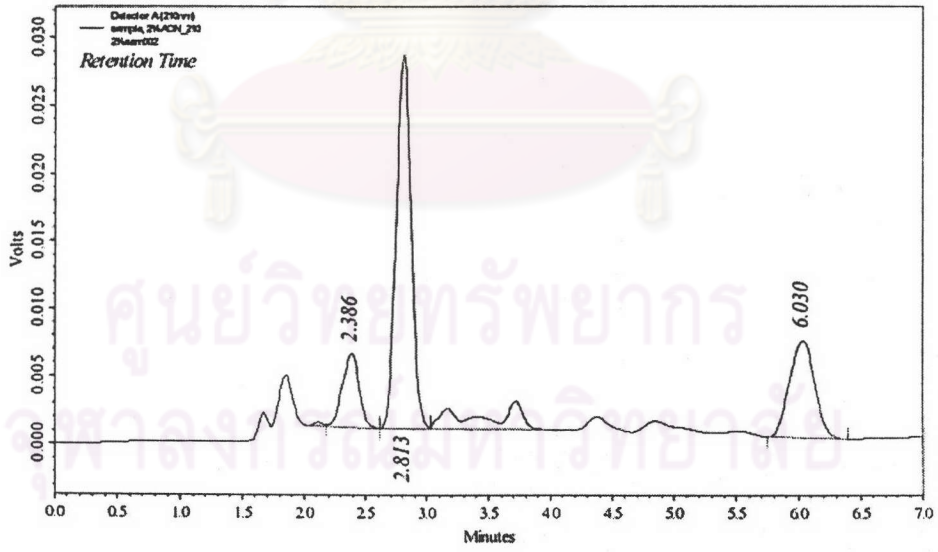


Figure 4 The chromatographic fingerprint of *H. sabdariffa* aqueous extract

H. sabdariffa aqueous extract was dissolved with 0.1 % phosphoric acid at the concentration of 0.60 mg/ml and 20 µl of the solution was injected into HPLC. The condition of HPLC was described in Materials and Methods.

2. Determination of total phenolic compounds using UV spectrophotometry

Total phenolic compounds in *H. sabdariffa* aqueous extract was determined spectrophotometrically using Folin-Denis reagent. Catechin standard curve was constructed between amount of catechin (0, 0.2018, 0.4036, 0.8072, 1.2108, 1.6144 mg) used and their corresponding absorbance. The correlation coefficient (r^2) of the standard curve was 0.9977 with the regression equation of y = 0.4911x + 0.0122 (Figure 34). Total phenolic compounds in the aqueous extract of *H. sabdariffa* was determined by performing the reaction and measured spectrophotometrically in the same manner as the reference standard. Amount of total phenolic compounds in the extract was calculated using the regression equation. Percentage of total phenolic compounds in the aqueous extract of *H. sabdariffa* was shown to be 3.874 % w/w thus the total phenolic compounds in the dried calyx of *H. sabdariffa* was subsequently estimated to be 0.958 % w/w (The detail of calculation was shown in appendix page 120).

Effects of *H. sabdariffa* aqueous extract on body weight, food and water consumption, liver weight and % relative liver weight

H. sabdariffa aqueous extract was given orally to rats at the doses of 250 and 1,000 mg/kg/day for 30 days. At both doses, the extract did not cause any significant effects on body weight gain, relative food consumption and relative water consumption (Figure 5-7). Liver weight and percent relative liver weight were also not affected by *H. sabdariffa* aqueous extract given at both doses to rats (Table 6).

Effects of *H. sabdariffa* aqueous extract on clinical blood chemistry and hematology

As compared to the control rats, subacute exposure (30 days) of oral 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract did not cause any significant effects on clinical blood chemistry. These parameters in serum included ALT (Figure 8), AST (Figure 9), ALP (Figure 10), total and direct bilirubin (Figure 11), total protein concentration, albumin and globulin (Figure 12), BUN and SCr (Figure 13), total cholesterol and TG (Figure 14), LDL-C and HDL-C (Figure 15), glucose (Figure 16), uric acid (Figure 17), calcium, sodium, potassium and chloride (Figure 18). Similarly, no effect of *H. sabdariffa* aqueous extract was observed on

these following hematological parameters: Hb and Hct (Figure 19), RBC count (Figure 20), RBC indices (MCV, MCH, MCHC) (Figure 21), platelet count and WBC count (Figure 22), % differential WBCs (Figure 23) and RBC morphology.

Effects of *H. sabdariffa* aqueous extract on hepatic CYPs

H. sabdariffa aqueous extract, given orally to rats at 250 and 1,000 mg/kg/day for 30 days, did not cause any significant changes of hepatic total CYP contents (Figure 24). The extract at both dosages also did not demonstrate any significant effects on the activities of ethoxyresorufin O-dealkylase (EROD) which represented the activity of CYP1A1, methoxyresorufin O-dealkylase (MROD) which represented the activity of CYP1A2, benzyloxyresorufin O-dealkylase (BROD) & pentoxyresorufin O-dealkylase (PROD) which represented the activity of CYP2B1/2 (Figure 25-28) as well as aniline 4-hydroxylase which represented the activity of CYP2E1 (Figure 29). CYP3A activity, which was examined by the rate of erythromycin N-demethylation reaction, was also not affected by *H. sabdariffa* aqueous extract (Figure 30).

Table 6 Effects of *H. sabdariffa* aqueous extract on body weight, liver weight and % relative liver weight

	Treatment group		
	Control group	*H. sabdariffa* group I	*H. sabdariffa* group II
Initial body weight (g)	312.39 ± 13.52	328.98 ± 15.65	304.56 ± 6.42
Terminal body weight (g)	380.31 ± 11.65	384.17 ± 14.30	344.88 ± 7.17
Liver weight (g)	12.38 ± 0.62	13.06 ± 1.03	11.67 ± 0.63
% relative liver weight (g/100 g of body weight)	3.25 ± 0.11	3.38 ± 0.19	3.32 ± 0.18

Data expressed as mean ± SEM

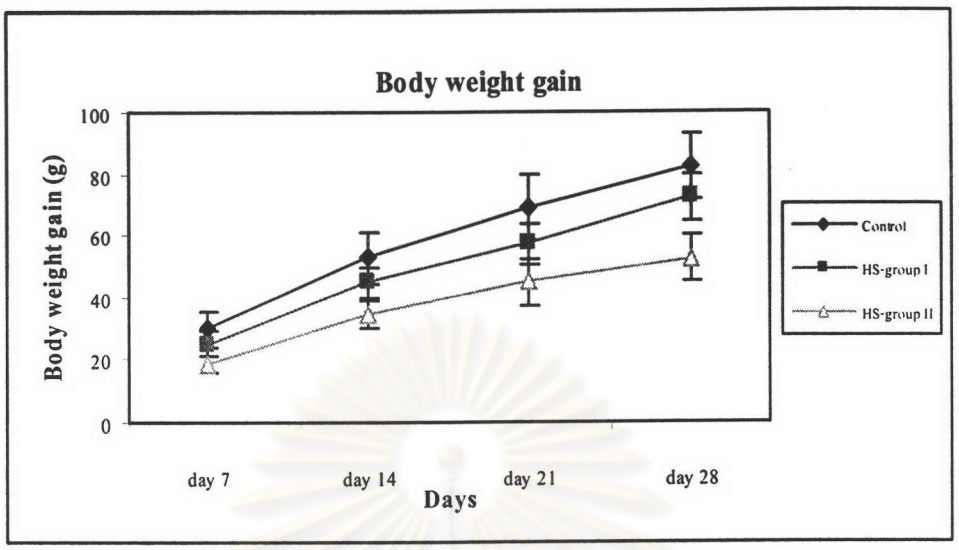


Figure 5 Subacute effects of *H. sabdariffa* aqueous extract on body weight gain of rats.

One millilitre/kg/day distilled water (Control, n = 10), 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9 respectively) were given orally to rats for 30 days. The individual mark represented the mean of body weight gain with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

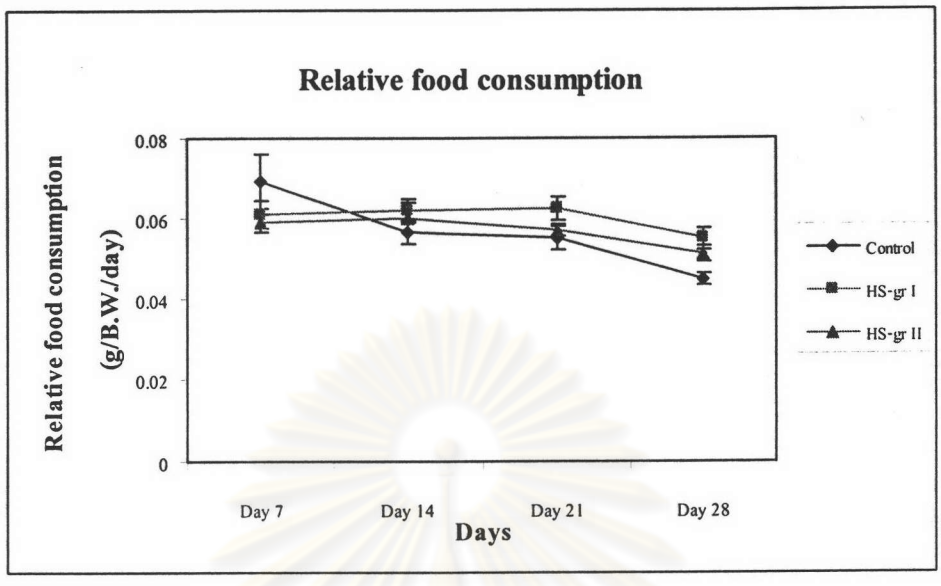


Figure 6 Subacute effects of *H. sabdariffa* aqueous extract on relative food consumption of rats. One millilitre/kg/day distilled water (Control), 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II, respectively) were given orally to rats for 30 days. Food consumption of each rat was recorded every 5 days. The individual mark represented the mean of relative food consumption per day with standard error of mean (SEM) (n = 10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

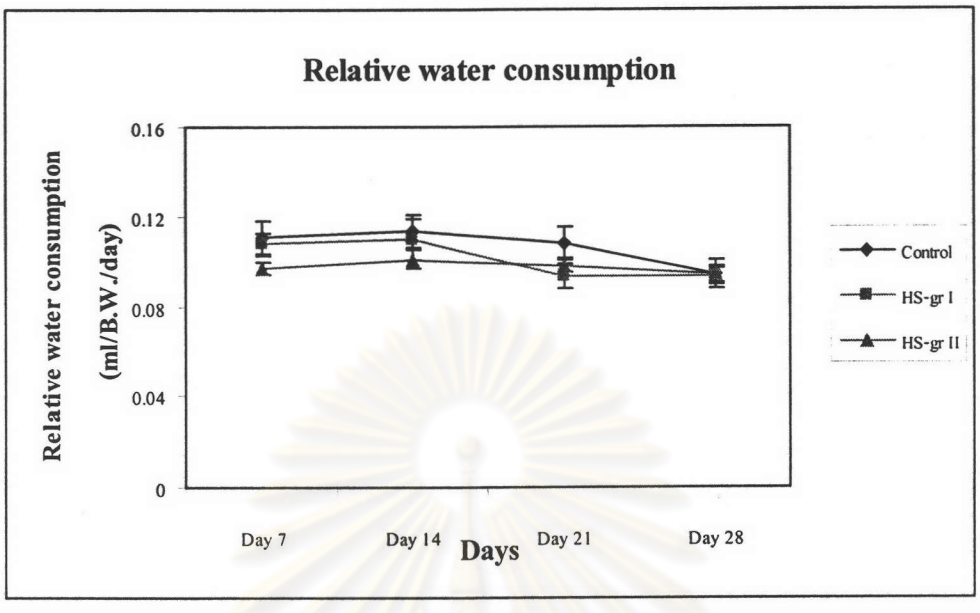


Figure 7 Subacute effects of *H. sabdariffa* aqueous extract on relative water consumption of rats.

One millilitre/kg/day distilled water (Control), 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II, respectively) were given orally to rats for 30 days. Water consumption of each rat was recorded every 5 days. The individual mark represented the mean of relative water consumption per day with standard error of mean (SEM) (n = 10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

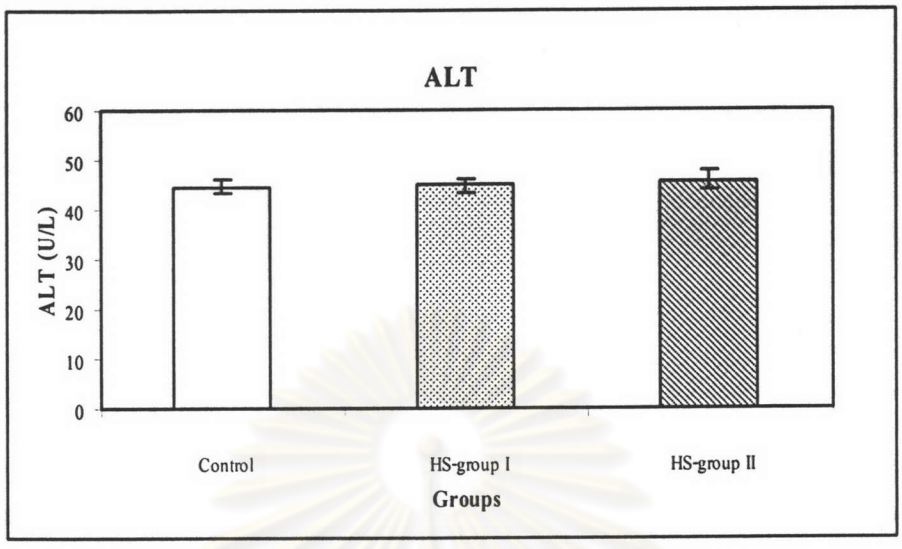


Figure 8 Subacute effects of *H. sabdariffa* aqueous extract on serum ALT.

One millilitre/kg/day distilled water (Control, n = 10), 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9 respectively) were given orally to rats for 30 days. Serum samples were determined for ALT concentrations. The individual bar represented the mean of ALT concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

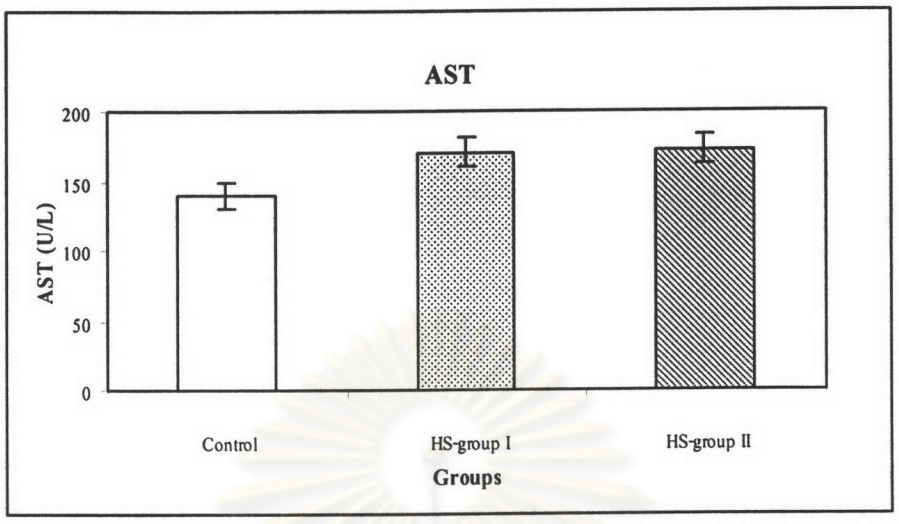


Figure 9 Subacute effects of *H. sabdariffa* aqueous extract on serum AST.

One millilitre/kg/day distilled water (Control, n = 10), 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9 respectively) were given orally to rats for 30 days. Serum samples were determined for AST concentrations. The individual bar represented the mean of AST concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

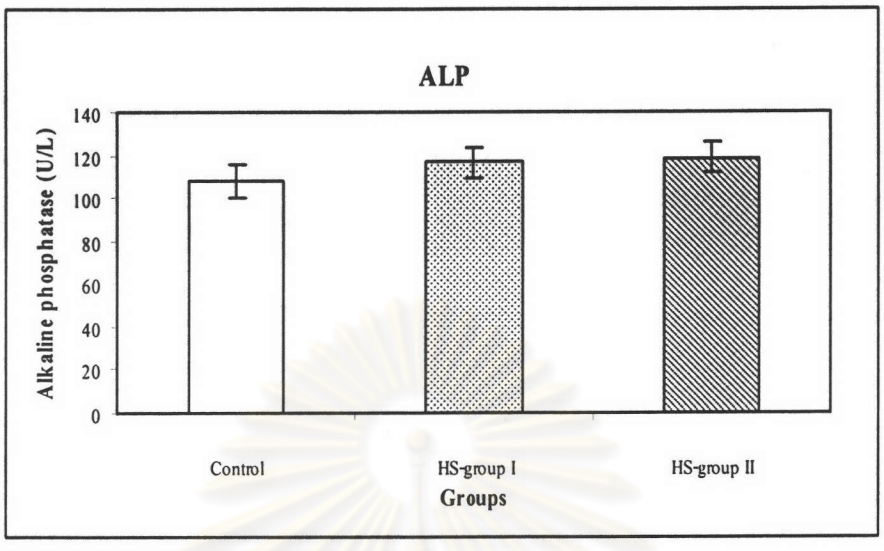
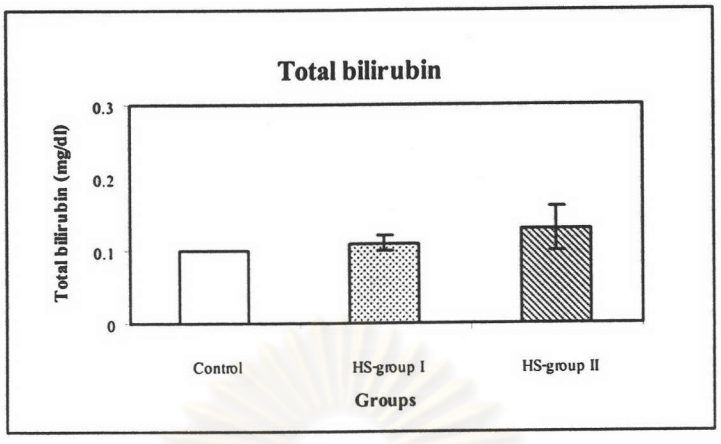


Figure 10 Subacute effects of *H. sabdariffa* aqueous extract on serum ALP.

One millilitre/kg/day distilled water (Control, n = 10), 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9 respectively) were given orally to rats for 30 days. Serum samples were determined for ALP concentrations. The individual bar represented the mean of ALP concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.



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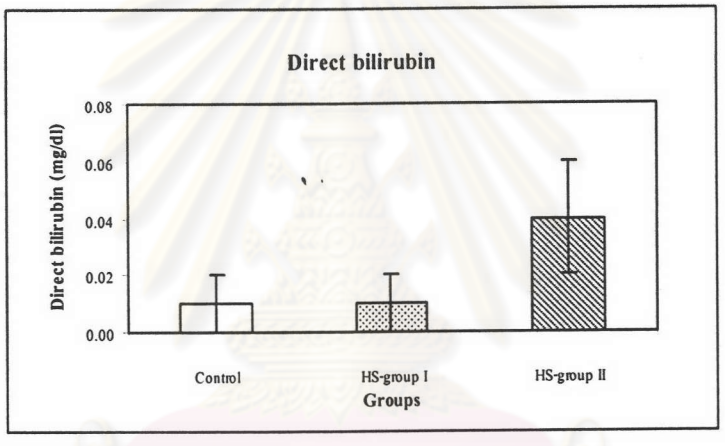
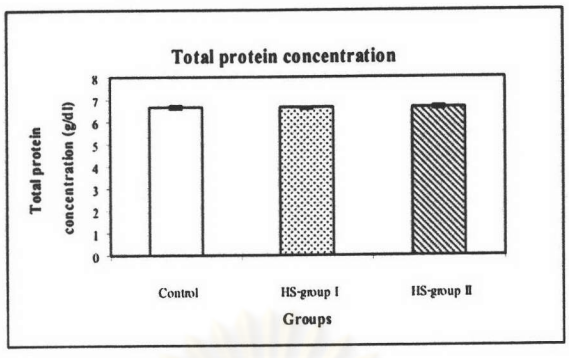


(b)

Figure 11 Subacute effects of *H. sabdariffa* aqueous extract on serum total bilirubin (a) and direct bilirubin (b).

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II respectively) were given orally to rats for 30 days. Serum samples were determined for total bilirubin (a) and direct bilirubin (b) concentrations. The individual bar represented the mean of serum bilirubin with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.



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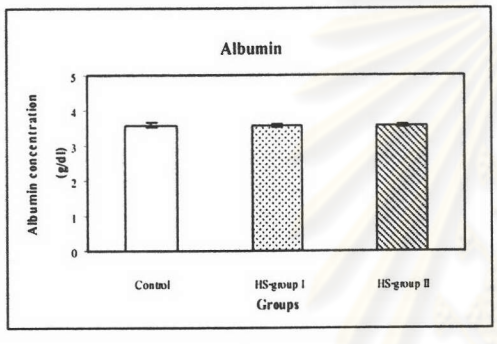

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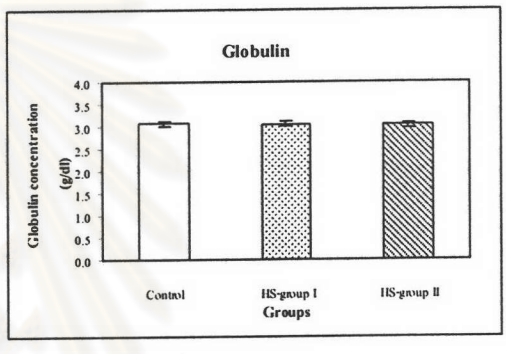
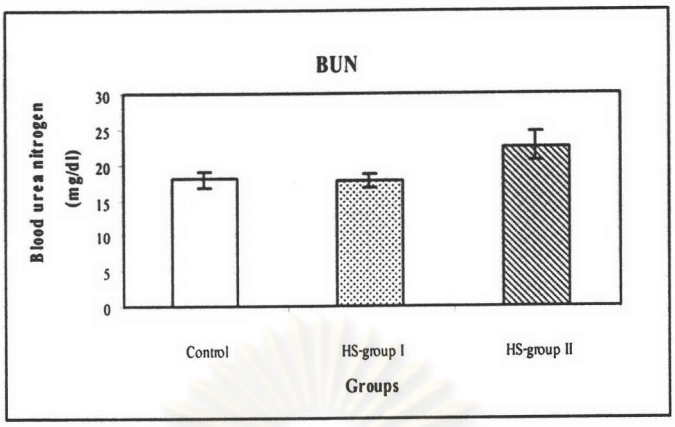


(c)

Figure 12 Subacute effects of *H. sabdariffa* aqueous extract on serum total protein (a), albumin (b) and globulin (c) concentration.

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II respectively) were given orally to rats for 30 days. Serum samples were determined for total protein (a), albumin (b) and globulin (c) concentrations. The individual bar represented the mean of serum total protein (a), albumin (b) and globulin (c) concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.



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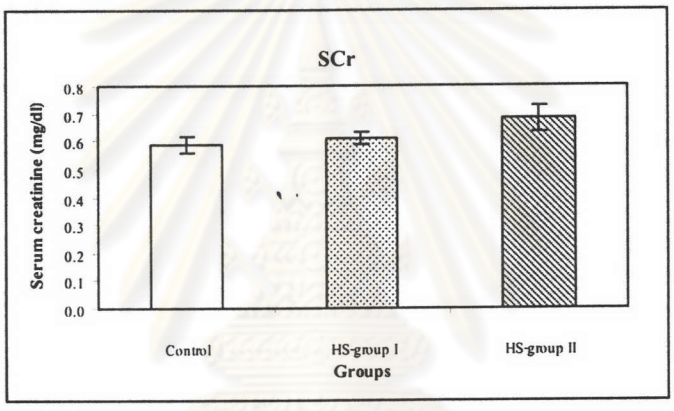
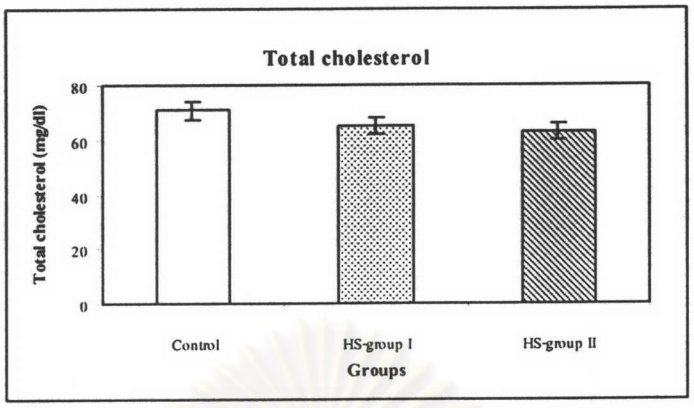


(b)

Figure 13 Subacute effects of *H. sabdariffa* aqueous extract on BUN (a) and SCr (b).

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II respectively) were given orally to rats for 30 days. Serum samples were determined for BUN (a) and SCr (b) concentrations. The individual bar represented the mean of BUN (a) and SCr (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.



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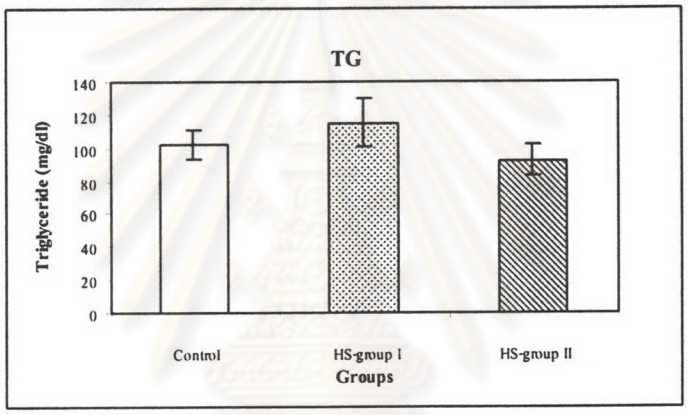
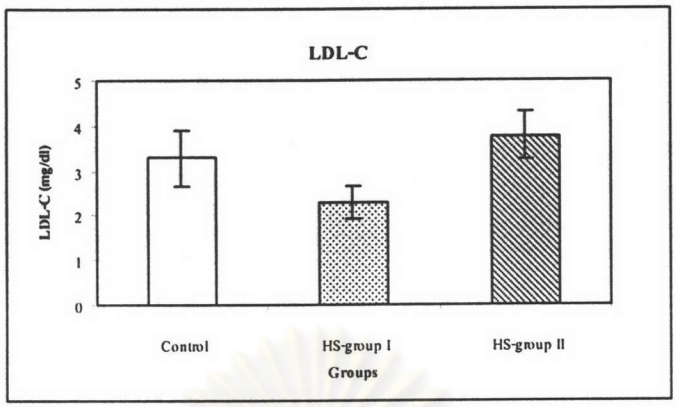


(b)

Figure 14 Subacute effects of *H. sabdariffa* aqueous extract on serum total cholesterol (a) and triglyceride (b).

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for total cholesterol (a) and triglyceride (b) concentrations. The individual bar represented the mean of total cholesterol (a) and triglyceride (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.



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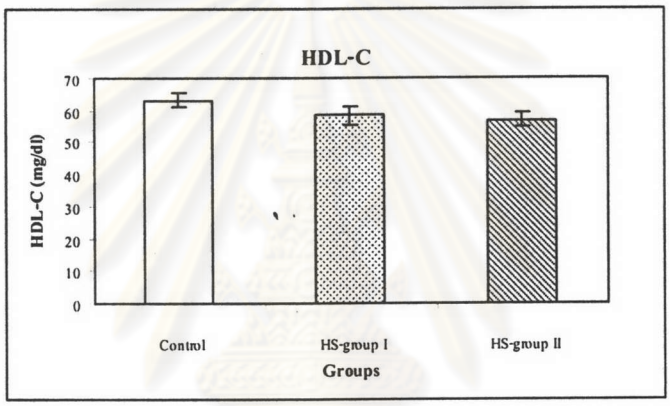


(b)

Figure 15 Subacute effects of *H. sabdariffa* aqueous extract on serum LDL-C (a) and HDL-C (b).

One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9 respectively) were given orally to rats for 30 days. Serum samples were determined for LDL-C (a) and HDL-C (b) concentrations. The individual bar represented the mean of LDL-C (a) and HDL-C (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

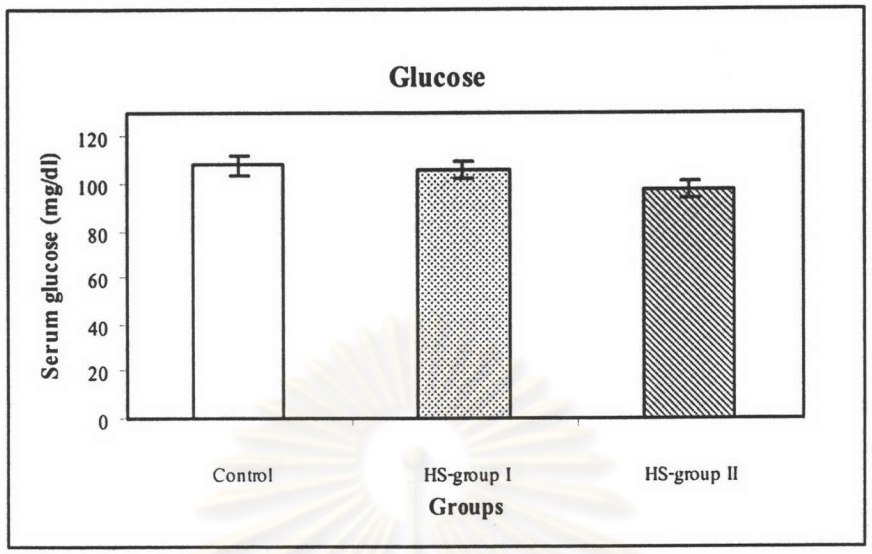


Figure 16 Subacute effects of *H. sabdariffa* aqueous extract on serum glucose.

One millilitre/kg/day distilled water (Control, n = 10), 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9 respectively) were given orally to rats for 30 days. Serum samples were determined for glucose concentrations. The individual bar represented the mean of glucose concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

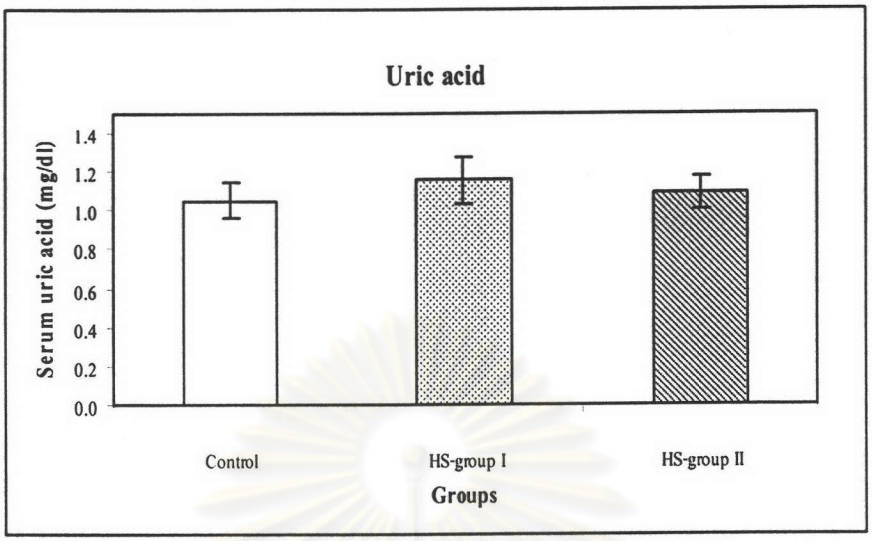


Figure 17 Subacute effects of *H. sabdariffa* aqueous extract on serum uric acid concentration. One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for uric acid concentrations. The individual bar represented the mean of serum uric acid concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

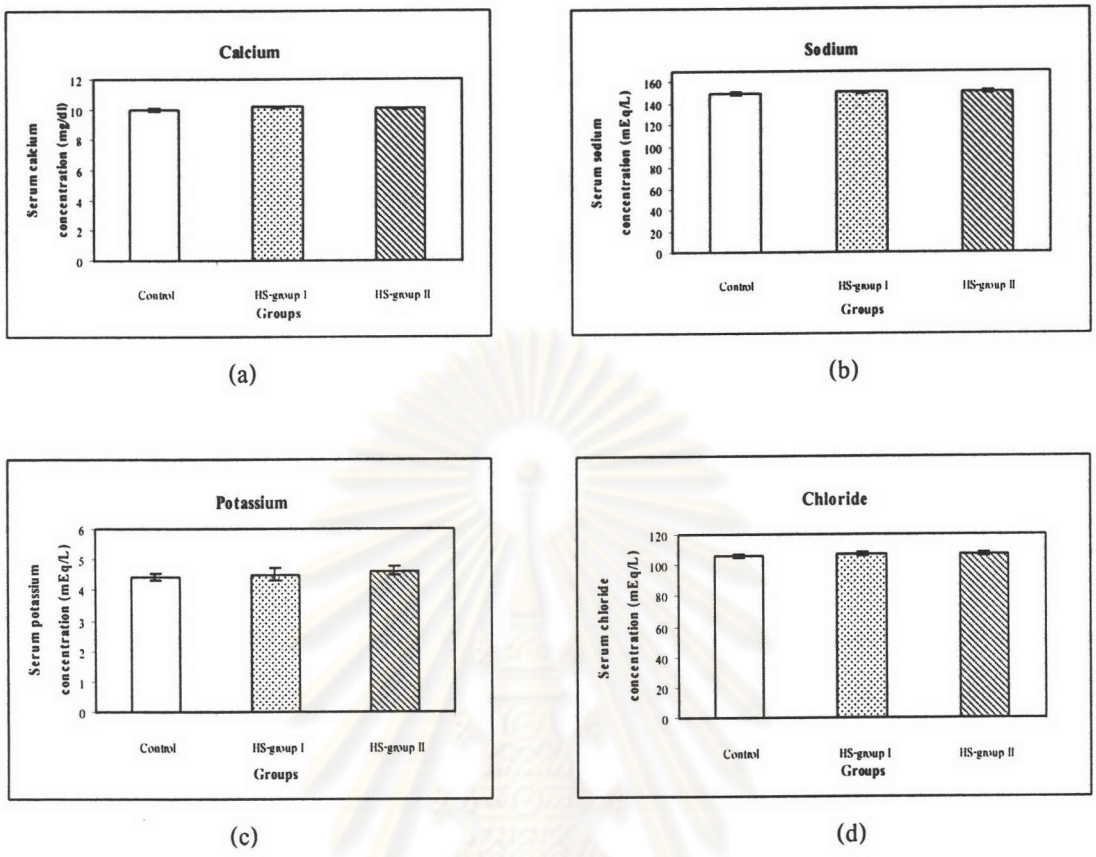
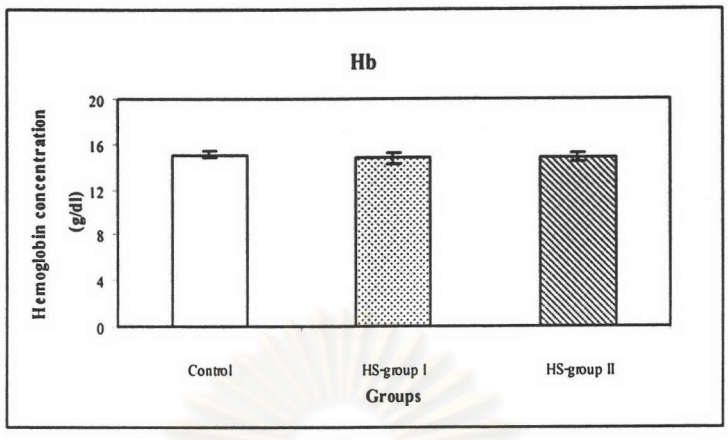
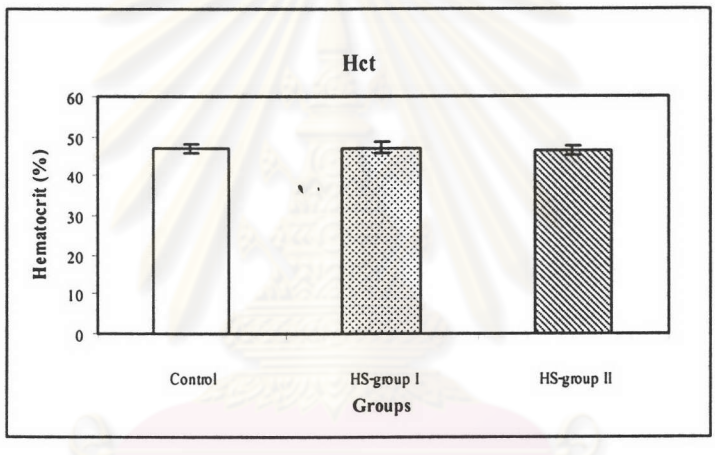


Figure 18 Subacute effects of *H. sabdariffa* aqueous extract on serum calcium (a), sodium (b), potassium (c) and chloride (d) concentration.

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for calcium (a), sodium (b), potassium (c) and chloride (d) concentrations. The individual bar represented the mean of serum calcium (a), sodium (b), potassium (c) and chloride (d) concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.



(a)



(b)

Figure 19 Subacute effects of *H. sabdariffa* aqueous extract on Hb (a) and Hct (b).

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II respectively) were given orally to rats for 30 days. Blood samples were determined for Hb (a) Hct (b) . The individual bar represented the mean Hb (a) Hct (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

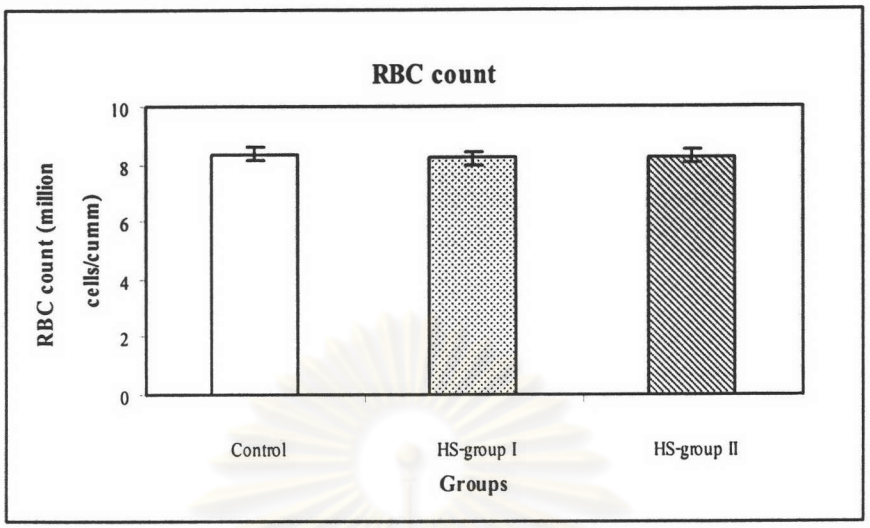


Figure 20 Subacute effects of *H. sabdariffa* aqueous extract on RBC.

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II, respectively) were given orally to rats for 30 days. Blood samples were determined for RBC count. The individual bar represented the mean of RBC count with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

RBC indices

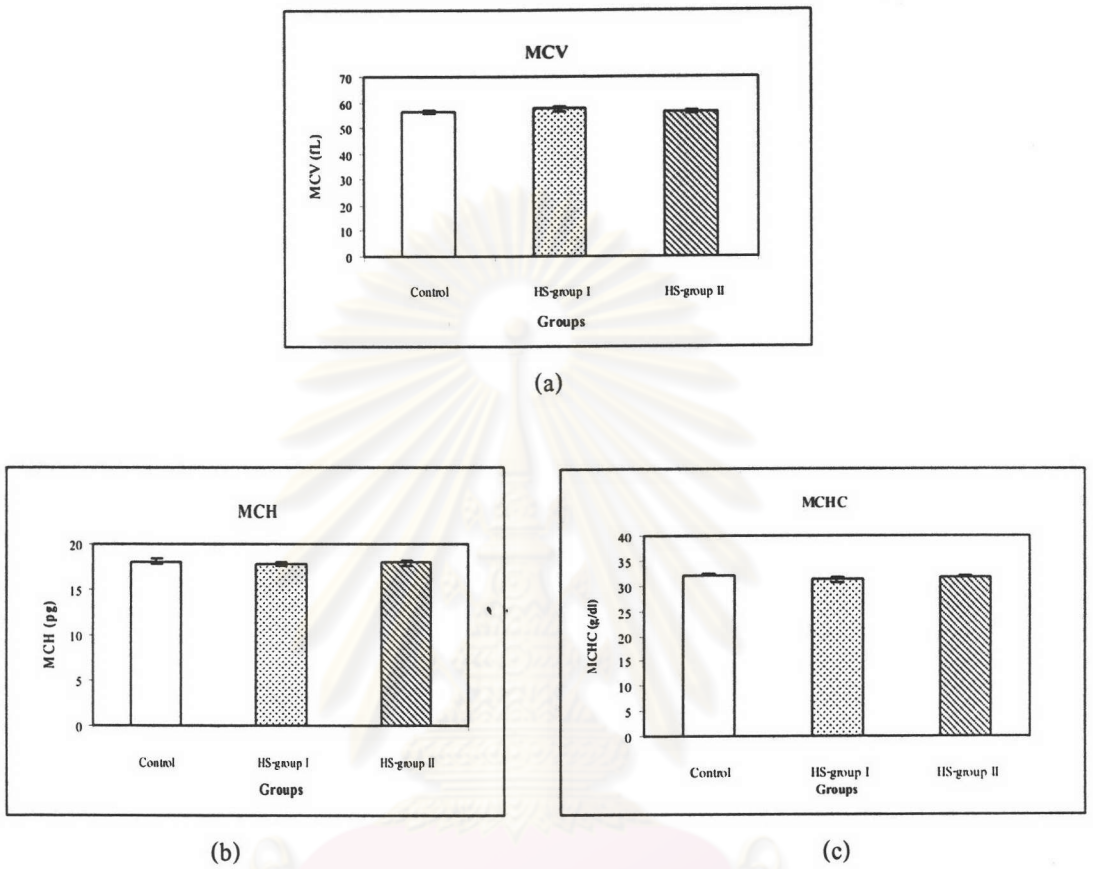
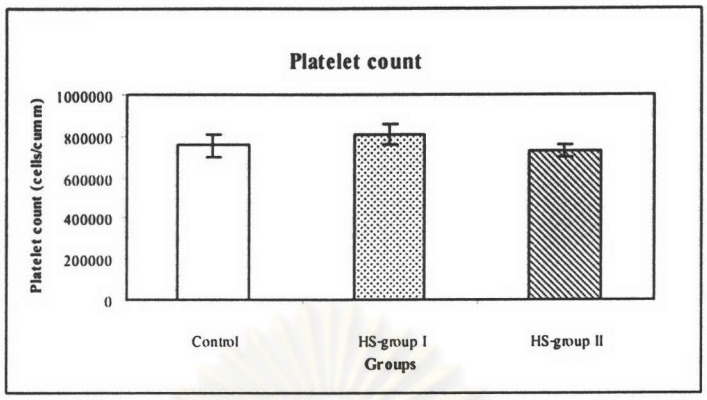
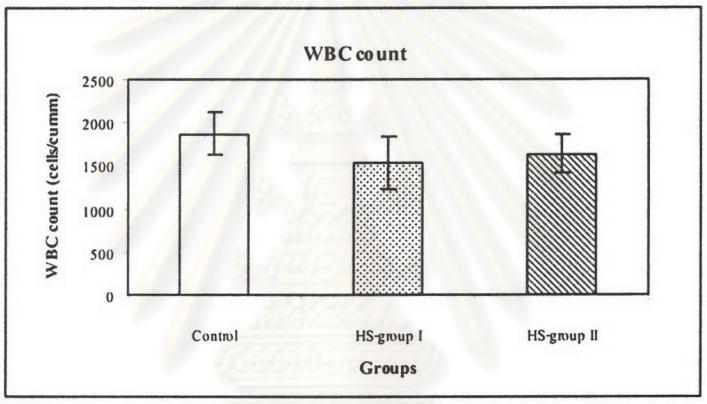


Figure 21 Subacute effects of *H. sabdariffa* aqueous extract on RBC indices.

One millilitre/kg/day distilled water (Control) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I & HS-group II, respectively) were given orally to rats for 30 days. Blood samples were determined for MCV (a), MCH (b), MCHC (c). The individual bar represented the mean of MCV (a), MCH (b), MCHC (c) with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.



(a)



(b)

Figure 22 Subacute effects of *H. sabdariffa* aqueous extract on platelet count (a) and WBC count (b).

One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9 respectively) were given orally to rats for 30 days. Blood samples were determined for platelet count (a) WBC count (b) concentrations. The individual bar represented the mean of platelet count (a) and WBC count (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

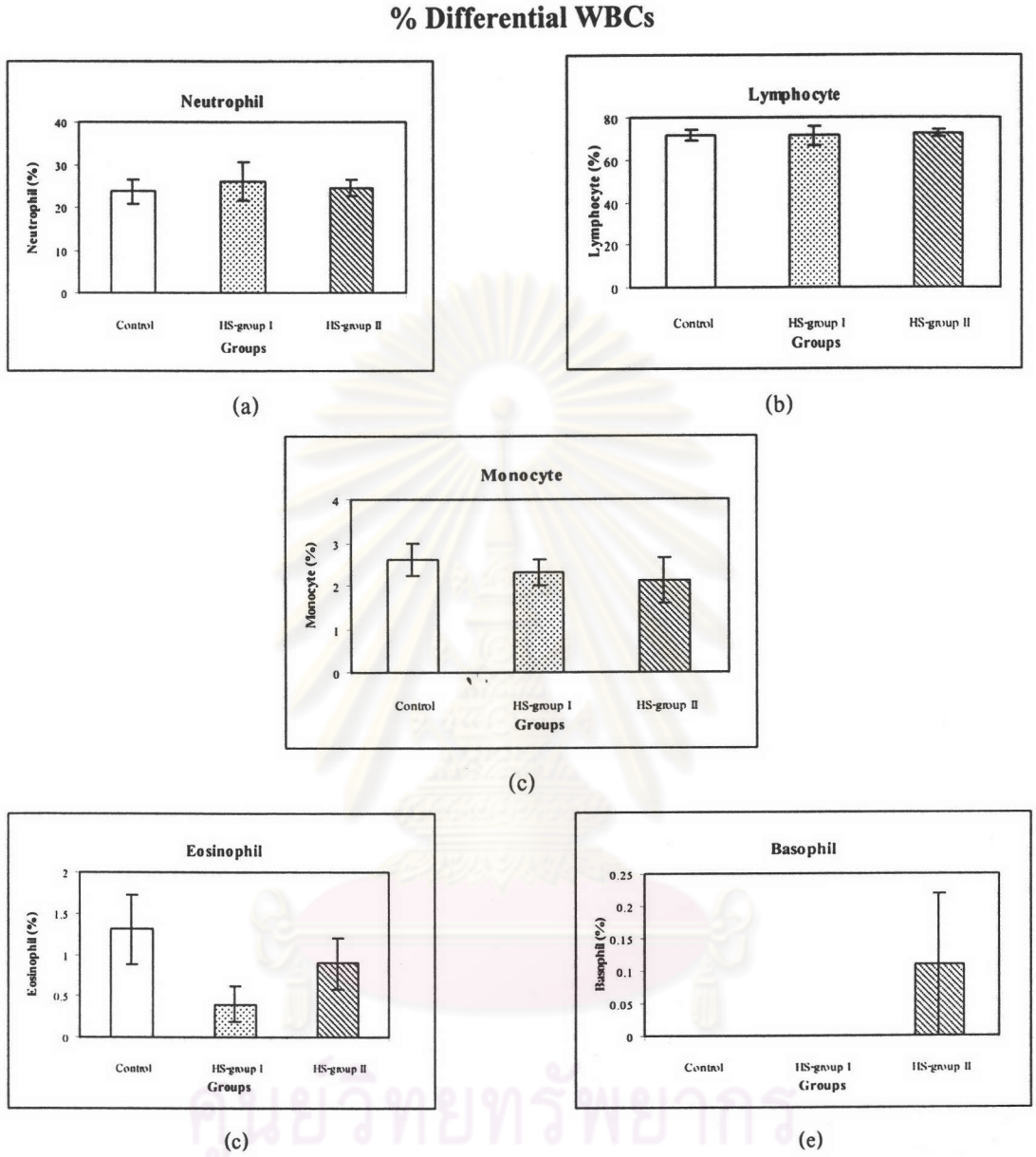


Figure 23 Subacute effects of *H. sabdariffa* aqueous extract on % differential WBCs.

One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Blood samples were determined for % differential WBCs. The individual bar represented the mean of % differential WBCs that included neutrophil (a), lymphocyte (b), monocyte (c), eosinophil (d) and basophil (e) with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

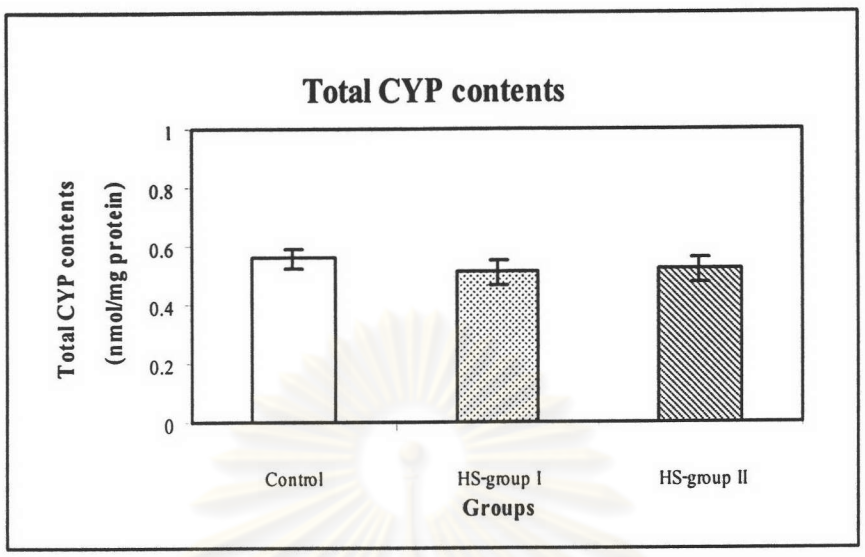


Figure 24 Subacute effects of *H. sabdariffa* aqueous extract on rat hepatic total CYP contents.

One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Liver microsomes were determined for total CYP contents. The individual bar represented the mean of total CYP contents with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

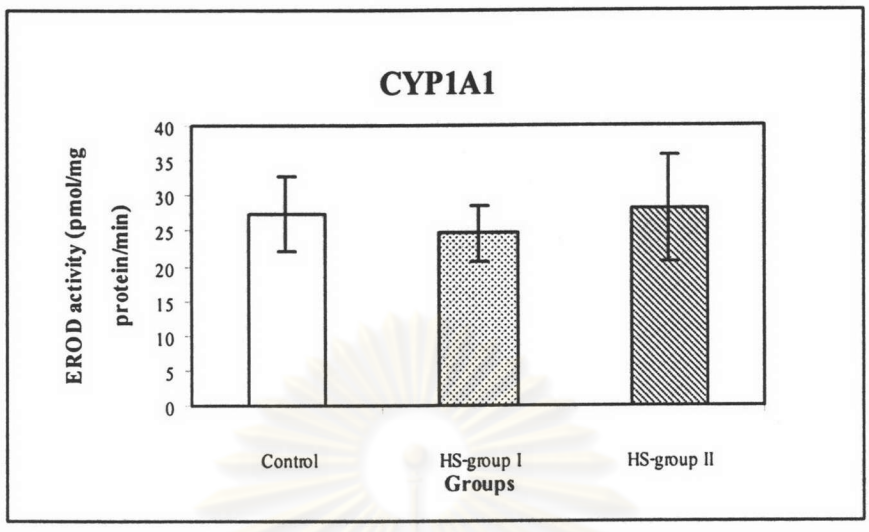


Figure 25 Subacute effects of *H. sabdariffa* aqueous extract on rat hepatic CYP1A1 activity. One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Liver microsomes were determined for EROD activity. The individual bar represented the mean of EROD activity with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

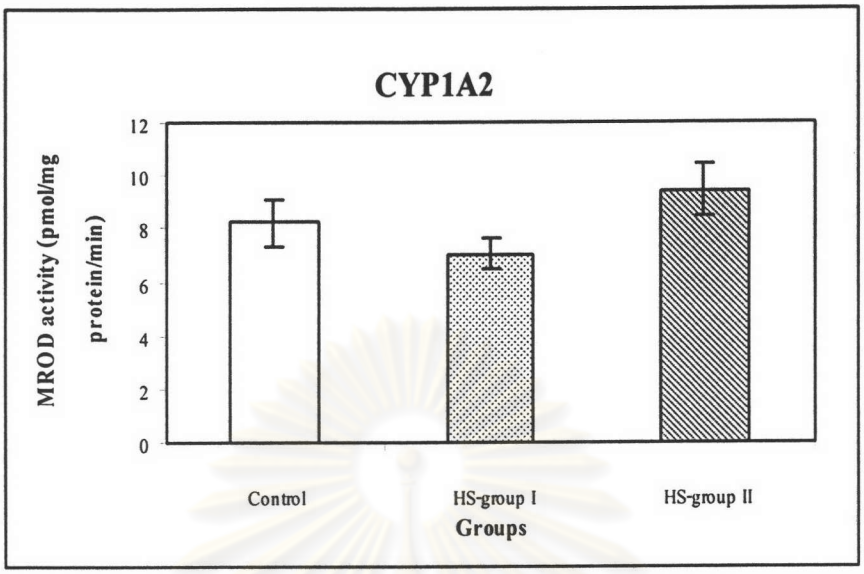


Figure 26 Subacute effects of *H. sabdariffa* aqueous extract on rat hepatic CYP1A2 activity. One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Liver microsomes were determined for MROD activity. The individual bar represented the mean of MROD activity with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

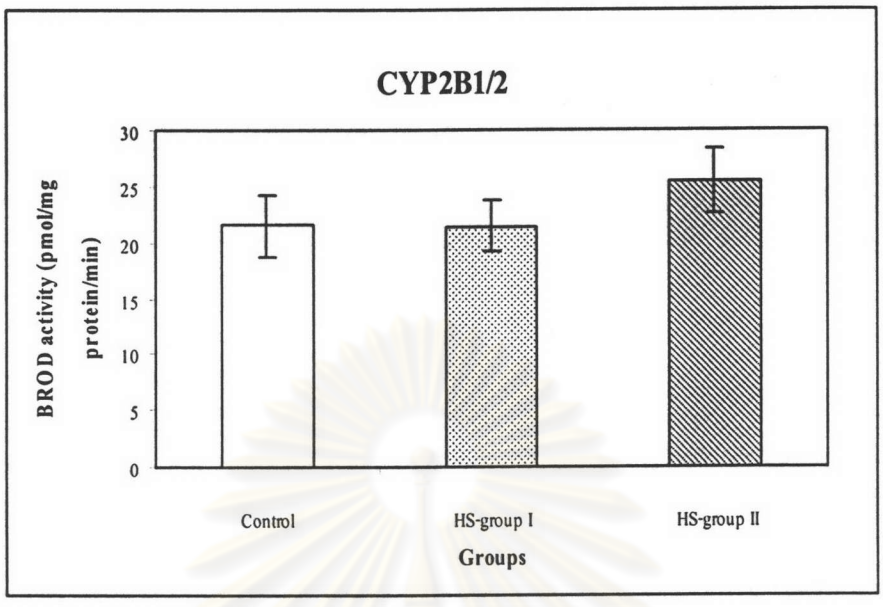


Figure 27 Subacute effects of *H. sabdariffa* aqueous extract on rat hepatic CYP2B1/2 (BROD) activity.

One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Liver microsomes were determined for BROD activity. The individual bar represented the mean of BROD activity with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

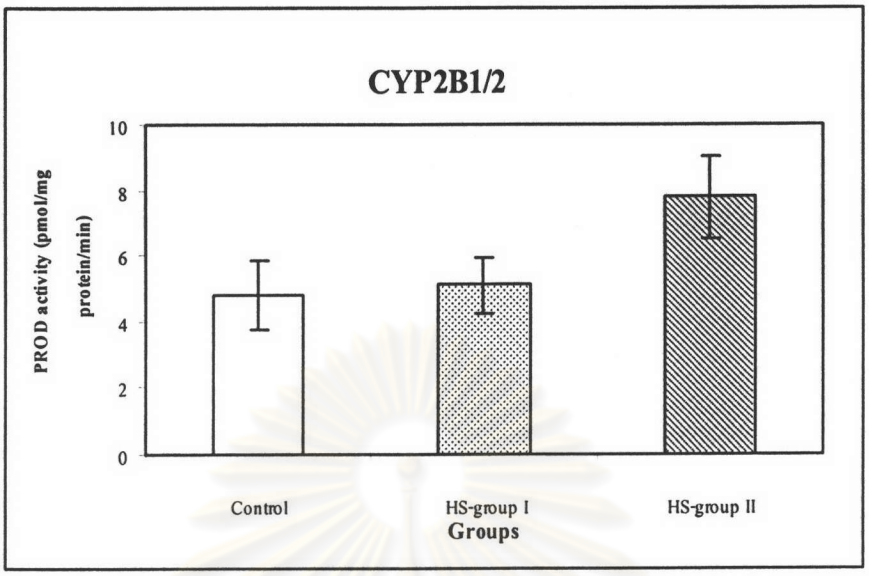


Figure 28 Subacute effects of *H. sabdariffa* aqueous extract on rat hepatic CYP2B1/2 (PROD) activity.

One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Liver microsomes were determined for PROD activity. The individual bar represented the mean of PROD activity with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

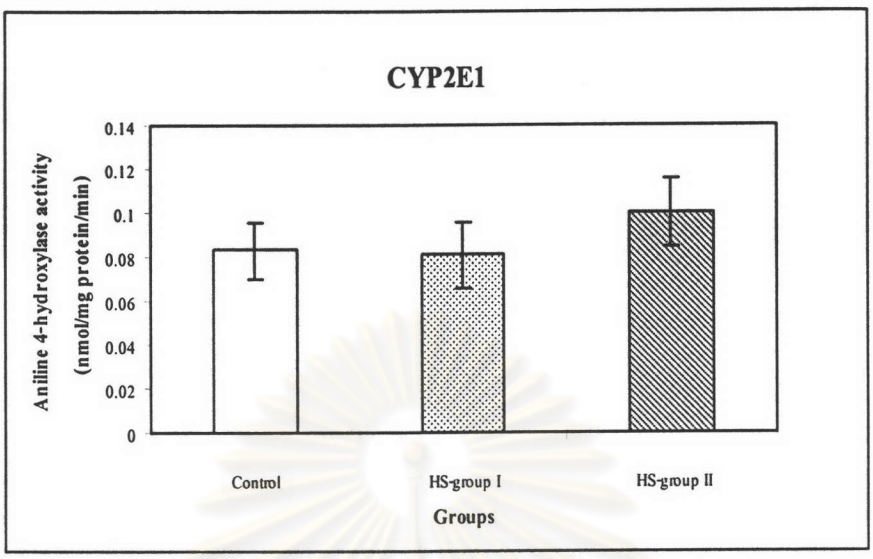


Figure 29 Subacute effects of *H. sabdariffa* aqueous extract on rat hepatic CYP2E1 activity. One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Liver microsomes were determined for aniline 4-hydroxylase activity. The individual bar represented the mean of aniline 4-hydroxylase activity with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.

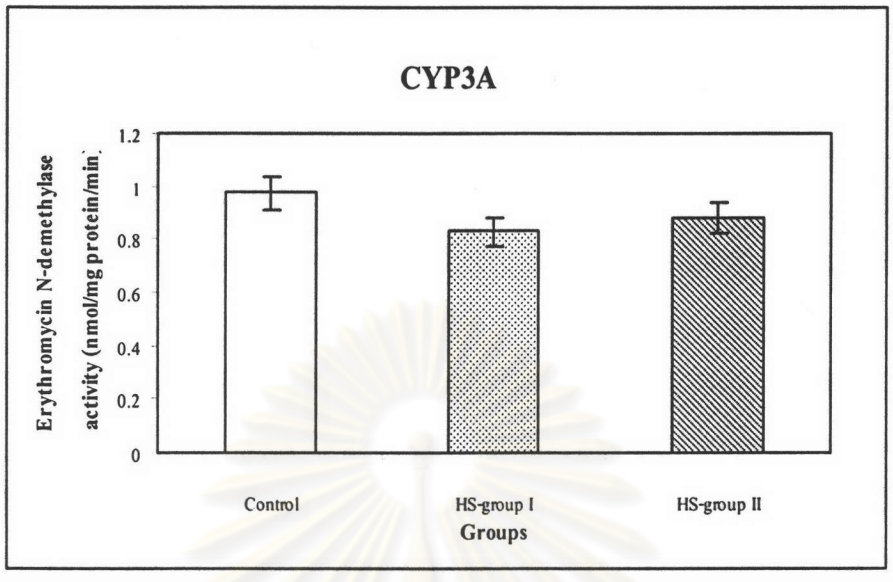


Figure 30 Subacute effects of *H. sabdariffa* aqueous extract on rat hepatic CYP3A activity. One millilitre/kg/day distilled water (Control, n = 10) 250 and 1,000 mg/kg/day of *H. sabdariffa* aqueous extract (HS-group I, n = 10 & HS-group II, n = 9, respectively) were given orally to rats for 30 days. Liver microsomes were determined for erythromycin N-demethylase activity. The individual bar represented the mean of erythromycin N-demethylase activity with standard error of mean (SEM). One-way ANOVA and Studen-Newman-Keuls test were used statistical comparisons at a significant level of $p < 0.05$.